REMARKS

Status of the Claims

Claims 1-2, 4, and 6-31 are pending. Claim 4 has been amended and new claims 20-31 have been added. Claims 7-15 have been withdrawn. Support for the amended and new claims can be found throughout the specification and in the claims as originally filed, for example, at paragraphs [017], [021], and [046], and original claim 4. Applicants respectfully submit that the amended and new claims do not constitute new matter.

Advisory Action

Applicants appreciate the Examiner's consideration and entry of the amendment and response filed on March 6, 2007. *See* Advisory Action.

Claim Rejections - 35 U.S.C. § 112, First Paragraph

The Advisory Action maintained the rejection of claims 1-2, 4, 6, and 16-19 under 35 U.S.C. § 112, 1st paragraph as set forth in the Office Action.

Applicants respectfully disagree and traverse this rejection.

In its last response, Applicants identified an example in the specification that teaches a method of proliferating cardiomyocytes *in vivo*. *See* Amendment and Response filed on March 6, 2007 at page 6. In Example 5, two kinds of adenoviruses (Ad-D1NLS and Ad-CDK4) were injected into the apical region of rat heart. As a control, an adenovirus comprising the *lacZ* gene was used. Four days after injections, the hearts were fixed and sections of tissues were stained with anti-Ki-67 antibodies. In the images of heart sections co-infected with the two vectors (Ad-D1NLS and Ad-CDK4), the Ki-67 nuclear protein, which is expressed in proliferating cells in all phases of the cell cycle, was expressed in cardiomyocytes. In the control containing the *lacZ* gene, however, the expression of the Ki-67 nuclear protein was not observed. These results, "strongly suggest that nuclear import of cyclin D1 and CDK4 could promote cell cycle entry of cardiomyocytes in adult hearts" and that "cardiomyocytes obtained the ability of proliferation by the expression of cyclin D1/CDK4 in the nucleus." *See* Example 5.

The Advisory Action states that this example is not enabling for all *in vivo* work. *See* Advisory Action at 2.

Applicants attach herewith the Declaration of Uichi Koshimizu, Ph.D. Under 37 C.F.R. § 1.132 ("Declaration of Dr. Koshimizu") providing further evidence that the specification enables an *in vivo* method of proliferating cardiomyocytes.

In his declaration, Dr. Koshimizu demonstrates that the introduction of adenoviral vectors expressing a D-type cyclin, CDK4 and a nuclear localization signal into adult rat cardiomyocytes *in vivo* proliferates the cardiomyocytes. *See* Declaration of Dr. Koshimizu at ¶¶ 7, 13-20, and 26.

Dr. Koshimizu describes the introduction of recombinant adenoviruses (Ad-CDK4 and Ad-D1NLS) into the heart. *Id.* at ¶¶ 13-16. To determine whether adult cardiomyocytes can undergo cell division *in vivo*, for example, the presence of H3P-positive cardiomyocytes after manipulation was investigated. *Id.* at ¶ 20. H3P-positive cardiomyocytes with characteristics from early prophase through telophase were identified in the D1NLS group, whereas no mitotic cells were found in a Control group, suggesting D1NLS and CDK4 expression promoted karyokinesis of adult cardiomyocytes *in situ*. *Id.* at ¶ 20 and Fig. 2. Quantitative analysis showed that the percentages of H3P-positive cardiomyocytes were 0 ± 0.0 % and 0.24 ± 0.07 % for Cont and D1NLS groups, respectively. H3P-positive cardiomyocytes were also observed in D1NLS group at 7 days after operation. *Id.* at ¶ 20. Furthermore, in the D1NLS group, cardiomyocytes at anaphase were also identified, expressing Aurora B in a cleavage furrow, and in some cardiomyocytes, Aurora B and Survivin were detected on the midzone between two daughter cells generated, indicating that these cells were undergoing cytokinesis. *Id.*; *see also id.* at Fig. 3. From the data obtained, Dr. Koshimizu concludes that CDK4 and D1NLS gene expression *in vivo* cause cell division of adult cardiomyocytes. *Id.* at ¶ 20.

Dr. Koshimizu also demonstrates that expression of D1NLS and CDK4 genes had protective effects on cardiac dysfunction and heart failure. *Id.* at ¶¶ 21-26. In particular, an echocardiographical analysis revealed that Fractional Shortening of left ventricular inner diameter (FS) of a D1NLS group was significantly higher than that of a Control group, suggesting that the expression of D1NLS and CDK4 expression protected ischemic hearts from left ventricular dysfunctioning. *Id.* at ¶ 22 and Table 1. Furthermore, in an experiment comparing the level of cardiac infarction in a heart six weeks post-ischemia and reperfusion, the results showed that the infact area in the D1NLS group was significantly reduced as compared to a Control group. *Id.* at ¶¶ 24-25 and Fig. 4.

In Dr. Koshimizu's opinion, a person of skill in the art would understand that the instant application teaches one of skill in the art to carry out a method for proliferating cardiomyocytes comprising introducing an adenoviral vector comprising a D-type cyclin and cyclin dependent kinase gene into cardiomyocytes *in vivo*. *Id.* at ¶ 27.

The Advisory Action states that Applicants only teach methods using adenoviral vectors. *See* Advisory Action at 2.

Applicants respectfully disagree and maintain that the specification teaches methods of using adenoviral and non-adenoviral vectors. Indeed, while the specification suggests that adenoviral vectors are preferred, the specification teaches that viral vectors such as retrovirus, vaccina virus, chick poxvirus and papovirus (e.g., SV40) vectors may be used. *See* paragraph [046]. Applicants note that claim 31 is directed to the use of a viral vector.

In view of the foregoing, Applicants respectfully request withdrawal of the enablement rejection under 35 U.S.C. § 112, 1st paragraph.

CONCLUSION

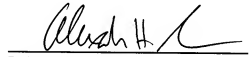
It is believed that these amendments and remarks should place this application in condition for allowance. A notice to that effect is respectfully solicited. If the Examiner has any questions relating to this response or the application in general she is respectfully requested to contact the undersigned so that prosecution of this application may be expedited.

It is believed that no fees are required for entry of this response, but should any fees be necessary, the Commissioner is authorized to charge such fees to the undersigned's **Deposit Account No. 50-0206**.

Respectfully submitted,

HUNTON & WILLIAMS, LLP

Dated: July 6, 2007

By: 

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